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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

The Determination of Morphine in Opium by Extraction: A New Method

In continuation of the international programme of scientific research undertaken pursuant to resolution 246 F (IX), adopted by the Economic and Social Council on 6 July 1949, the Secretary-General is now publishing a new method for the determination of morphine in opium which has been worked out by the Secretariat.

The Secretary-General wishes to call attention to the fact that the morphine analysis is useful not merely in helping to determine the origin of contraband opium seized from the illicit traffic; it is of paramount importance in the commercial and medical evaluation of licit opium, and in national and international accounting of opium used and drugs produced. It is believed that the Secretariat's new method provides a more accurate standard for the determination of morphine in opium. The Secretary-General suggests, therefore, that it be studied in comparison with the present pharmacopoeial methods to establish the accuracy or degree of error of the latter; and also studied as an assay procedure in its own right.

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/THE DETERMINATION OF

THE DETERMINATION OF MORPHINE IN OPIUM BY EXTRACTION: A NEW METHOD

I. SYNOPSIS OF METHOD

1. Stage I: The opium is ground in a mortar with lime and water.

Stage II: The alkaline strength of the filtered lime-water extract is reduced with ammonium chloride and the solution extracted continuously with ether-acetone (a new solvent combination for this purpose) until all the morphine is out. The resulting extract is evaporated to dryness in a dish.

Stage III: The residue is transferred to a separatory funnel with chloroform and dichloracetic acid solution, and partitioned by shaking out. Dichloracetic acid solutions in three separatories retain the morphine while chloroform takes out all the other alkaloids. (An entirely new method of separating the other opium alkaloids from morphine.)

Stage IV: The dichloracetic acid solutions are then combined, made ammoniacal, and the morphine shaken out with chloroform-isobutanol and chloroform-ethanol. The organic solvent extracts are washed and filtered into a dish and then evaporated to dryness.

Stage V: The residue of crystalline morphine is dissolved in a little methyl alcohol, an excess of tenth-normal acid added, using methyl red indicator; the solution diluted with water and back titrated with tenth-normal alkali.

In sum: with the exception of Stage I, (the extraction of the opium with lime-water, for which an aliquot is taken) the extractions are made complete by test. From Stage II onward, it is thought that any negative or positive error in determining the morphine is kept within the equivalent of about 0.1 cc of tenth-normal acid; and thus, it is believed that the objective is essentially realized of separating all the morphine and finally titrating all of it and nothing else.

II. DISCUSSION

2. It is nearly a century and a half since morphine, the first-discovered alkaloid, was originally isolated from opium, but there is still no general agreement as to any uniform "best method" for determining the percentage of morphine in different kinds of opium, nor is any commonly accepted method entirely free from the possibility of substantial, irherent errors. Dozens of chemists have written hundreds of articles on this subject without completely

/solving the

solving the problem. It is, however, believed that the present method is at least a substantial contribution to the subject and indeed in large part a solution of the problem.

3. In an assay, the separation of morphine from lime-water or other aqueous solution, and from the other alkaloids, may be accomplished either by precipitation or by extraction with organic solvents. For the assay of raw opium, precipitation methods are commonly used commercially, and are official in the pharmacopoeias. The "International Method", devised under the auspices of the League of Nations and later accepted by some pharmacopoeias, is such a method. Extraction methods are also used officially in certain cases, when precipitation methods are almost inapplicable; for example, in the assay of camphorated tincture of opium.

4. In spite of the present predominance of precipitation methods for the analysis of opium itself, extraction methods have major points of superiority, assuming that the special difficulties that have beset them can be overcome. Precipitation methods are not flexible: even without considering opium preparations, but only the assay of unmixed raw opium, the results are far from being equally dependable on all types of opium. Furthermore, it is never possible to precipitate all the morphine from a solution; hence a correction must be applied, usually an arbitrary one, or a balancing of errors must be relied on. The precipitated morphine, in the usual methods, is by no means free from other alkaloids. In contrast, an extraction method has a much greater possibility of being applicable to different types of opium with widely varying percentages of morphine, and even to many preparations and mixtures; the extractions can be made complete; and only by an extraction method can we hope to attain the ideal of titrating all the morphine and nothing but morphine.

5. The principal objections that have been brought against previous extraction methods are the following:

(a) That, due to the very limited solubility of morphine in most organic solvents, the sample of opium taken is rather small, increasing the difficulty of getting a representative sample and usually increasing the proportionate effect of impurities.

(b) That in spite of the small sample, large volumes of solvent are used, and the shaking-out of the morphine is excessively difficult and tedious.

/(c) That the

(c) That the residue is frequently too highly coloured.

(d) That, although the non-phenolic alkaloids are separated, the minor phenolic alkaloids remain with the morphine.

6. In the present procedure the opium equivalent (4 grammes) is the same as for the precipitation method of the United States Pharmacopoeia. By means of continuous extraction for Stage II, the objection (a) is met completely and objection (b) in large part. The use of ether-acetone meets objection (c), for this new solvent combination extracts far less colour and other impurities than do solvent-combinations previously used, such as benzene-isopropanol or chloroform-ethanol.

7. The objection (d) that the minor phenolic alkaloids are included with the morphine in extraction processes, although often conceded, has not been nearly as true as has been assumed. Chloroform, as a matter of fact, extracts most of the minor phenolic alkaloids, as well as the non-phenolic alkaloids, from alkali solution. Although this has not been generally recognized, most extractive procedures have utilized back-extraction of the morphine from solution of the alkaloids in chloroform-isopropanol, or some corresponding procedure which separated the greater part of the other phenolic alkaloids from the morphine. When some solvent such as benzene was used to remove the non-phenolic alkaloids from alkali solution the error may have been considerable; with the use of chloroform it can scarcely have exceeded about 0.3 percent of the opium. (This is not to say that previous extraction methods necessarily gave too high results, for there may have been compensating errors in the other direction.) In any case the new method uses instead an extraction from acid which seems to be completely successful.

8. The back-extraction of morphine from an organic solvent solution by means of dilute acid has been used in some previous extractive methods, though probably not for opium assays. Usually it accomplishes little in the way of separating alkaloids; it may or may not result in some separation of the other titratable alkaloids from the morphine, depending on the solvent and the acid in question. In the present procedure, dichloracetic acid has been chosen as the only acid yet found which, with chloroform, will accomplish the separation desired, and which even makes unnecessary the separation using aqueous alkali.

9. Theoretically, the steps of the new method subsequent to Stage I could be carried out just as well if the aqueous extraction of the opium were made with

/acidified

acidified water instead of lime-water (the solution afterward being made sufficiently alkaline to free the morphine for extraction). In practice there are some difficulties which require further study. Objections have been raised against the use of lime-water for the opium assay, but the most successful and generally accepted methods use it. It has seemed best to work out the rest of the procedure first, and thus provide a sound basis for comparison of this method with the pharmacopoeial and similar assays. In making comparisons, the procedure in Stage I can be modified to correspond exactly, if desired, to the pharmacopoeial assay with which comparison is made. The extraction method has the advantage, however, that there is not the same necessity for limiting the volume of the lime solution, as for a precipitation method.

10. The Secretariat chemists gladly acknowledge a general indebtedness to previous workers in this field. No list of references is given in the present paper; since it is assumed that the interested chemists are acquainted with the basic methods for opium assay, and it is hoped that an extensive bibliography, with commentary, can be offered later.

III. THE METHOD

Stage I. Extraction of the opium with lime-water

11. In a large mortar with pouring lip, grind up 6 grammes of the opium with 20 cc water and 3 grames calcium hydroxide, $Ca(OH)_2$. Add 70 cc additional water and stir frequently during half an hour, making sure that finally no opium masses remain undisintegrated. If a sticky layer forms on the pestle, scrape it off with a knife if necessary, and regrind. Pour the lime-water extract through a folded filter and collect 61 cc, representing 4 grammes opium (allowance of 1 cc for extractives).

Stage II. Extraction of the alkaloids from the opium extract

12. The solvent used consists of 150 cc of ethyl ether, $(C_2H_5)_2O$, and 100 cc of acetone, $(CH_3)_2CO$. Introduce the lime-water extract to be analyzed, with 250 cc of ether-acetone solvent (150 + 100), into a continuous extraction apparatus (such as that illustrated in Annex A to document E/CN.7/195) with an accompanying boiling-and-receiving flask of 250 to 500 cc capacity; at the same time adding 1.75 grammes ammonium chloride, NH_4Cl, to the lime-water extract. Perhaps the best way is to put a little of the NH_4Cl in the apparatus first, in case a little of the lime-water extract runs through the porous plate. Fill

/the apparatus

the apparatus below the plate with the ether-acetone before pouring in the lime-water extract, and have some of the ether-acetone solvent running into the reception tube of the apparatus, through a folded filter paper, while the extract is poured into the main tube. This procedure will usually keep all of the aqueous solution above the porous plate. Add the rest of the NH_4Cl and rinse in the last drops of the extract, washing down any NH_4Cl clinging to the walls of the main tube, with a few cc water. Keep some ether-acetone solvent running in at the same time. Pour the rest of the ether-acetone solvent into the boiling-and-receiving flask, add a few grains of silicon carbide, SiC, to promote even boiling; connect the apparatus, and turn on the electric hot-plate and the water.

If the porous plate becomes clogged during the extraction, so that the ether-acetone does not rise through it readily enough, the condition is usually corrected easily by adding 2 or 3 cc water down the solvent delivery tube. This can be done through the condenser while the boiling continues.

13. Extract until the morphine is all out. This usually requires at least 4, more often 5 hours, and sometimes considerably longer. To determine if the morphine is all out, shut off the heat, stop or greatly reduce the boiling, and withdraw a test portion of the solvent - about 1 cc - from above the aqueous extract. Evaporate it in a small white porcelain crucible and text with Concentrated Marquis reagent, 9 cc concentrated H_2SO_4 + 1 cc formaldehyde solution (37 - 40%). If the test is not distinctly positive for morphine, another test or tests may be tried, using 3 to 4 cc of the solvent solution at a time, and, if desired, Froehde's and Mecke's reagents as well as Marquis'. (Froehde's reagent: 0.5 gramme sodium or ammonium molybdate in 100 cc concentrated H_2SO_4. Dissolve with the aid of heat. Contrary to statements in much of the literature, it will keep indefinitely in a glass-stoppered bottle. Mecke's reagent: 0.5 gramme selenious acid in 100 cc concentrated H_2SO_4.) If the test for morphine still coming out is very weak, and especially if the continuation of the extraction has to go over to the next day, it is best to pour the extract already obtained into an evaporating dish (see the following step, #14), and use a fresh portion of 50 to 150 cc of ether-acetone (60 + 40 ratio) to finish the extraction. In any case, whether the boiling is restarted with the same solvent or fresh solvent, <u>any time the boiling has been completely stopped, add a few grains of SiC before starting it again</u>.

/14. When the test

14. When the test shows no more morphine coming out, pour the liquid in the receiving flask into an evaporating dish weighed to the nearest centigramme. Rinse the receiving flask into the dish with a little methanol; if any morphine has crystallized out in the flask, use hot methanol and rinse two or three times. Evaporate to dryness and then heat for half an hour on the steam bath, in order to drive off volatile bases if any are present. Cool and weigh the dish with its contents before proceeding to the next step. (This weighing is not essential but may yield useful information.)

Stage III. Separation of the other alkaloids from morphine

15. Treat the residue of alkaloids with 15 cc chloroform, $CHCl_3$; cover the dish with a watch-glass, and heat on the steam bath just until the chloroform is hot. Take from steam bath, and, keeping under the hood to avoid breathing the $CHCl_3$ vapors, loosen the residue with a glass rod. Pour the solution, with as much of the residue as possible, into a separatory funnel.

16. Repeat with 10, 7 1/2, 7 1/2, 5, and 5 cc of $CHCl_3$, thus transferring the residue to the separatory funnel with $CHCl_3$. (It may not always be necessary to use so many separate portions. A total of 50 cc $CHCl_3$ is used.) Now add 0.5 cc of pure dichloracetic acid to the dish. This will dissolve any brownish residue still sticking to the inside of the dish. Add 5 cc water and pour into the same separatory funnel. Rinse out the dish twice more with 5 cc water plus a few drops of dichloracetic acid each time. Pour into the same separatory funnel.

In two more separatory funnels put 10 and 10 cc water, with two or three drops dichloracetic acid in each.

17. If any other alkaloids are to be determined from the same extract, two more separatories are necessary (fourth and fifth), containing respectively 8 cc of 5% NaOH solution and 5 cc of 2% NaOH solution. (See part VI for determination of "alkaloid 3" and of any morphine carried out of the acid solutions; and parts VII and VIII for determination of codeine or "porphyroxine-meconidine".) If only the routine determination of morphine is desired, it is only necessary to interpose a few cc of fresh 2% NaOH solution before each test for completion of the extraction (see paragraph 20).

18. Shake the first separatory thoroughly, and add a small piece of litmus to make sure that the aqueous layer remains acid. Withdraw the $CHCl_3$ and shake it with the other two dichloracetic acid solutions in turn; and, if any other

/alkaloids are

alkaloids are to be determined, with the two alkali solutions. The $CHCl_3$ is then saved for redistillation or for determination of codeine or "porphyroxine-meconidine".

19. Continue the shake-out with more portions of $CHCl_3$, 25 cc each. After five or six shake-outs test a small portion of the $CHCl_3$ for continued extraction of codeine. When the two alkali solutions are not used, the $CHCl_3$, withdrawn from the third acid solution, or part of it, is shaken with a few cc of fresh alkali solution and a small portion taken for the test. Evaporate the test portion in a small white porcelain crucible, cool, and test the residue with concentrated Marquis reagent. A purple colour indicates continued extraction of codeine. If desired, other tests may be made with Froehde's reagent, to be sure of the absence of morphine in the $CHCl_3$ tested, and with Mecke's reagent, which yields a green colour with codeine. When the two alkali solutions are used, test the $CHCl_3$ as it is withdrawn from the first alkali solution (fourth separatory). When the Marquis' indication of codeine has become very slight, shake a few cc of the next extract, upon withdrawing it from the last acid solution (third separatory) with a few cc of fresh alkali, and test this $CHCl_3$ portion with Marquis' and Mecke's reagents for continued extraction of codeine. (This procedure is used because there is a little hold-up of codeine, and more of some other alkaloid yielding a green colour with Mecke's reagent, in the main alkali solution.) The extra portion of alkali may be added to the main alkali solution.

20. Continue until the test for codeine being extracted has become negative or completely negligible. This usually requires some 8 to 14 portions of $CHCl_3$. (For further use of the alkali solutions see part VI. For further use of the $CHCl_3$ see parts VII and VIII.)

Stage IV. Final extraction of the morphine

21. Combine the acid solutions by pouring from the second separatory into the first, and from the third into the second and then into the first, so that all the dichloracetic solutions are in the first separatory. Then put 5 cc water in the third separatory, shake, and pour it into the second separatory (but not into the first). Put 5 cc more water in the third separatory, shake, and make it ammoniacal with a drop or two of concentrated NH_4OH, then pour it into the second separatory. Finally, add 5 cc water to the third separatory. See that the solutions in the second and third separatories are ammoniacal.

/22. Put in the

22. Put in the first separatory 50 cc of chloroform-isobutanol-methanol solution (30 + 15 + 5). Also have ready 100 cc of chloroform-isobutanol solution (75 + 25). Put a piece of litmus paper in the first separatory and add concentrated NH_4OH, a few drops at a time, until the solution is alkaline to litmus; then add about 1 cc in excess. At once shake vigorously.

23. As soon as there is a good separation and the layers have become nearly clear, withdraw the lower layer from the first separatory into the second, and shake the second separatory. Again, as soon as the layers are well separated, withdraw the lower layer into the third separatory and shake. Then, when the layers have separated, withdraw the lower layer from the third separatory, filtering it through a pledget of cotton in the neck of an ordinary funnel, into an accurately weighed dish which will hold 100 cc of solution.

24. At the same time continue the extraction with the 100 cc of chloroform-isobutanol solution, using 25 cc at a time. When the first two or three extracts (50 and 25, 25 cc) have been run into the dish, put it on the steam bath and evaporate until it will hold more extracts.

25. Run the remaining chloroform-isobutanol extracts into the dish and continue the shakeouts with chloroform-ethanol (85 + 15), 25 cc at a time. Evaporate a little of the first chloroform-ethanol extract from the main solution (first separatory) in a small white porcelain crucible and test the residue with Mecke's reagent. If no green colour is obtained, the aqueous solution in the first separatory can be discarded. (In cleaning this separatory, any sticky brown resinous material remaining in it is easily dissolved by alkali solution.) When the first solution is free of morphine by the Mecke test, a test is made on the extract from the second separatory (with the same or a fresh chloroform-ethanol solution), and finally on the extract from the third separatory. If morphine is still coming out, the extractions are continued with chloroform-ethanol; or if a considerable content of morphine still present is indicated, one or two extractions can be again made with chloroform-isobutanol (3 + 1, ratio), then again with chloroform-ethanol (85 + 15, ratio). (On the other hand, if the morphine content of the opium is known to be quite low, one or two of the chloroform-isobutanol extractions can be omitted, or the volume of the first solvent, the chloroform-isobutanol-methanol, can be somewhat reduced.) The chloroform-ethanol extracts are run into the same dish as the preceding extracts.

/26. Evaporate off

26. Evaporate off the solvent and dry the residue on the steam bath to complete crystallization. Heat for about half an hour more; cool in the desiccator and weigh. Multiplication of the weight by 0.94 gives an approximation of the content of anhydrous morphine.

Stage V. Titration of the morphine

27. Add 15 cc methanol to the residue, put a watch glass over the dish, and heat on the steam bath until the hot methanol has dissolved all the morphine. If necessary, break up any hard lumps with a stirring rod. (Occasionally, dispersed particles of impurities remain undissolved.) Add 4 or 5 drops methyl red indicator solution, and run in standard tenth-normal H_2SO_4, stirring with a glass rod. Note the approximate point at which the solution becomes permanently red; add 5 to 10 cc of tenth-normal H_2SO_4 in excess. See that all the residue is dissolved as completely as possible. If necessary, heat again to be sure all the morphine is in solution. (Especially with heating, which is not used unless necessary, there may be a little brownish precipitate of some impurity.) Dilute to about 100 cc with CO_2-free distilled water (recently boiled. Back-titrate with tenth-normal NaOH to disappearance of the red colour of the indicator. Ascertain that one drop, of if necessary two drops, of the tenth-normal H_2SO_4 will restore a slight red tint to the solution. This final acid is included in the acid reading. Subtract the amount of alkali used from the amount of acid used. Multiply the difference - the cc of tenth-normal acid used for neutralization of the morphine - by .0285 to obtain the weight of anhydrous morphine. (To calculate as crystalline morphine, multiply by .0303.) To convert to percentage, multiply by 100 and divide by the number of grammes of opium extracted for morphine (4 in this case).

IV. NOTES

Stage I. Extraction of the opium

28. In the "International Method" the opium is ground up with lime and water, rinsed into a flask and shaken for the aqueous extraction. In order to calculate an exact aliquot, determinations are made for moisture and for the extractives in the lime solution. The morphine is precipitated by adding NH_4Cl to the lime solution. The morphine titrated is equivalent to only about 2.5 grammes of the opium. A large abritrary correction is added.

/29. In the

29. In the method of the British Pharmacopoeia, the opium is ground up with water and the lime added to the mixture. A volume allowance is made for extractives. The morphine is precipitated by adding NH_4Cl to the lime solution. The morphine titrated is equivalent to 5 grammes of the opium. A correction is added for morphine remaining in solution.

30. In the method of the United States Pharmacopoeia a total extraction is first made with water. The filtered extracts are concentrated by evaporation, lime added, and a theoretically exact aliquot obtained. The morphine is precipitated by adding NH_4Cl to the lime solution. The morphine titrated is equivalent to 4 grammes of the opium. No correction is added. It is claimed that codeine and possibly other titratable alkaloids, precipitating with the morphine, are just about equivalent to the morphine remaining in solution, in the usual opium. However, it is generally conceded that the U.S.P. results are too low on opiums that are low in morphine.

31. Stage I of the present method is on the whole most like the B.P. method. The International and U.S.P. methods both involve considerable trouble to obtain an aliquot, which does not seem justified by the over-all accuracy in either case. Total extraction is also desirable but there is no absolute proof that all the morphine of the opium is finally in solution in the lime water, in the U.S.P. method. The B.P. method formerly allowed 1 cc for extractives from 5 grammes opium, now allows 2 cc for extractives from 5 grammes. The investigations of the Secretariat have been conducted upon opiums in the air-dried condition; the conservative allowance of 1 cc for extractives from 4 grammes of opium is considered satisfactory for these tests.

32. In all lime methods it is important that the lime be very near full strength. Because of insistence of a number of chemists on this point, the United States Pharmacopoeia formerly required the use of freshly-slaked lime, but this is no longer stated. The directions for the International Method include a method for testing the lime. (Bulletin of the Health Organization of the League of Nations, Vol. VII, Extract No. 6.) The usual $Ca(OH)_2$ for chemical purposes has generally seemed satisfactory in the present investigations.

Stage II. Extraction of the alkaloids from solution

33. Morphine is not highly soluble in ether-acetone. The great advantage of the new solvent combination is in extracting so little material besides the alkaloids. In spite of the fact that water is gradually taken out of the

/aqueous layer

aqueous layer into the solvent layer and the aqueous column sometimes goes far down in the extractor before the morphine is all out, very little colour and very little of other impurities are extracted, in comparison with the quantities taken out by other solvents or combinations capable of extracting morphine. Ether-acetone requires a rather close adjustment of the pH for sufficiently rapid removal of the morphine; this is accomplished in the present method. The NH_4Cl added may be varied from 1.5 to 2.0 grammes.

Stage III. Separation of the other alkaloids

34. Codeine could, of course, be separated from the morphine more easily by shaking out from an alkali solution; but then extraction from dichloracetic acid solution would still be required to remove the "alkaloid 3". It may be mentioned that the other alkaloids are even more readily extracted by chloroform from trichloracetic acid solution than from dichloracetic; but too much morphine is then carried along.

Stage IV. Final extraction of the morphine

35. A number of other alcohols can be used instead of isobutyl, and some further experiments with others will still be made. Tests resembling trace reactions for morphine persist for a number of shakeouts with Marquis' and Froehde's reagents, particularly with certain kinds of opium, after the Mecke reaction has become negative (no green colour). It is not even certain that these persisting reactions are due to morphine, and collection of additional extracts has added only an insignificant titration with fiftieth-normal acid (equivalent to less than 0.1 cc of tenth-normal). Therefore the cut-off in this stage is made with Mecke's reagent.

Stage V. Titration of the morphine

36. There are some coloured substances in the final residue, with greater discoloration in some cases than others, but it is fairly easy to make the titration within one drop, or at any rate within 0.1 cc, of tenth-normal acid.

V. POSSIBILITIES OF SUBSTANTIAL ERROR

Possibilities of negative error

37. Aside from spillage or an outright mistake in reading or recording some weight or volume, there seem to be three major possibilities of negative error (too low results). These are:

(1) Incomplete

(1) Incomplete extraction of the morphine from the opium by lime solution.

(2) Oxidation of morphine while in the lime-water solution or during the rather long extraction with ether-acetone and the subsequent evaporation of the solvent along with extracted water.

(3) Accumulation of small losses without any noticeable spillage.

38. The first of these possibilities is shared with any other method of lime-water extraction; indeed to some extent with any method of extraction. The new method in its subsequent stages provides for the first time a sound basis for comparing different methods of extracting the opium, but the working out of details is necessary.

39. The second possibility is believed not to occur, but this is not quite certain. There are other compounds in an opium solution even more easily oxidized than morphine, which protect the morphine from oxidation to a great degree.

40. That the third possibility may be sufficiently serious, is shown by difficulty in exactly checking a result by re-extraction of the morphine and retitration. The method requires some practice and experience; but this is probably true also of all other methods of opium assay.

Possibility of a positive error

41. Again aside from a mistake, the only major possibility of a positive error would seem to lie in the possibility of the extraction of some other titratable base. Obviously, such a base would have to be capable of being set free by ammonia and yet strong enough to be titrated, and at the same time not be capable of extraction from alkali. Although extraction from alkali is not used in the method, it can be applied after the titration, and it is found that the material finally present, extractable by chloroform from alkali solution, consists chiefly of residual traces of codeine and should not yield a titration of more than 0.5 cc of fiftieth-normal acid (0.1 cc of tenth-normal acid). In addition, such a hypothetical compound could not be very volatile nor highly soluble in water, nor could it be extracted by chloroform from dichloracetic acid solution. There is no present evidence for the existence in opium of any basic compound meeting all these qualifications, except the morphine. Conclusion: It is unlikely that there is any possibility of a substantial positive error in the new method.

VI. DETERMINATION OF "ALKALOID 3" AND OF MORPHINE CARRIED PAST THE ACID SOLUTIONS

42. Shake the alkali solutions from Stage III (part III, paragraphs 18-21) with a few portions of fresh $CHCl_3$, until the extractable alkaloids have been shaken out and a test on the residue from a small portion of the $CHCl_3$ gives no green colour, or at most a negligible one, with Mecke's reagent. Then acidify the second alkali solution with dichloracetic acid and pour it back into the first. Substitute 5 cc water with a few drops of dichloracetic acid in this separatory. Add more dichloracetic acid to the first alkali solution until it is acid. In two more separatories put 5 cc dilute NH_4OH solution and 5 cc water respectively.

43. Shake out with $CHCl_3$, 20 to 15 cc at a time, shaking each portion first with the main dichloracetic acid solution, then with the acid wash water, then with the ammoniacal solution, and lastly with the last wash water. Immediately after each shaking with the ammoniacal solution, test with a piece of moistened red litmus at the mouth of the separatory, to be sure that this solution remains ammoniacal, since some dichloracetic acid is carried along by the $CHCl_3$.

Filter the extracts into a dish. Evaporate a small portion of a $CHCl_3$ extract for test with Froehde's reagent. With "alkaloid 3" this yields a gray-green-blue colour, which on standing becomes an intense violet. Mecke's reagent yields a bright green which on standing becomes an intense violet; this is also characteristic but the Froehde reaction is the one to watch for any indication that morphine is beginning to come out instead of the "alkaloid 3" (purple-red colour replacing or preceding the first colour due to "alkaloid 3"). If the final wash solution becomes strongly coloured during the shake-out, pour it back into the ammoniacal solution and replace with 5 cc fresh water. Continue until the test for "alkaloid 3" being extracted is negligible. About 6 extractions are required.

44. Evaporate off the $CHCl_3$. Weigh the residue if desired. Dissolve it in a little methanol, add recently boiled water, and titrate with fiftieth-normal acid, using methyl-red indicator. A purer residue could be obtained as follows: Shake the $CHCl_3$ extracts of paragraph 43 with alkali solution, thus returning the "alkaloid 3" to alkali solution. (The $CHCl_3$ can be saved for redistillation.) Then wash the alkali solution with a little CCl_4, acidify it with HCl or (1 + 3) H_2SO_4, make ammoniacal, and shake out the "alkaloid 3"

/with CCl_4.

with CCl_4. The "alkaloid 3" may possibly be codamine, but this is far from certain; it may not be among the named alkaloids of opium.

45. When the "alkaloid 3" has been shaken out of the dichloracetic acid solutions, discard the solutions in the third and fourth separatories; pour the acid wash solution back into the main solution, replace with 3 cc water, make ammoniacal, pour this back into the main solution, and replace with 5 cc water. Make the main solution ammoniacal and shake out the morphine, if any appreciable amount is present, with portions of chloroform-ethanol solution (85 + 15). Shake each chloroformic solution with the wash water, then filter into a dish. Evaporate off the $CHCl_3$, weigh the residue if desired, dissolve in a little methanol, add recently-boiled water, and titrate with fiftieth-normal acid, using methyl red indicator.

46. Thus far it seems that the percentage of "alkaloid 3" does not vary much in different types of opium. The titration is about 2.0 – 2.5 cc of fiftieth-normal acid. In methods which utilize only a shake-out from alkali solution to separate the other alkaloids from morphine, this titration is included with that of morphine and means a positive error equivalent to about 0.3% of the opium. This titration is probably not wholly due to a single base, the "alkaloid 3", but it seems that most of it is.

47. The morphine that gets as far as the alkali solution is usually negligible, and its determination is chiefly important in allowing the analyst to check his technique. Neither the morphine extracted past the acid solutions, nor the non-morphine alkaloids remaining in the acid solutions, should show as much titration as 0.5 cc of fiftieth-normal acid.

VII. DETERMINATION OF CODEINE

48. The chloroform extracts from Stage III of the Method (part III, paragraphs 15-21) are used. In each of two separatory funnels put 5 cc of dilute H_2SO_4 (2 cc concentrated H_2SO_4 per 100 cc solution). Shake the combined chloroform solutions with first one, then the other, of these two solutions. (If the volume of the chloroform is too much for a separatory funnel at one time, it can be put through in two portions.) Run the chloroform solutions back into the flask in which they were collected.

49. Pour the acid solution in the second separatory funnel into the first one, then replace with 5 cc more of dilute H_2SO_4 (2 vol.%). Add 2 or 3 drops of

/concentrated

concentrated NH_4OH to the chloroform solution and shake. Test with a piece of litmus to see that the chloroformic solution is now ammoniacal. Then shake it out with the 5 cc of 2% H_2SO_4 in the second funnel. The chloroform is now reserved for redistillation.

50. Wash the aqueous solutions with two portions of CCl_4 of about 12 cc each. Discard this CCl_4 as it is mixed with $CHCl_3$. Then buffer the aqueous solutions, the first with 4 grammes and the second with 2 grammes of sodium acetate.

51. Shake out the thebaine with CCl_4. Continue as in the usual separation of thebaine and cryptopine from codeine (E/CN.7/202, paragraphs 10 and 11): Evaporate portions of the CCl_4 and test for thebain with (2 + 1) H_2SO_4. When the thebaine is nearly out, check on extraction of cryptopine with fuming H_2SO_4 (15% free SO_3), and on whether any codeine is coming out, with "(3 + 1)" Marquis' reagent (4 drops HCHO, 3 cc H_2SO_4, 1 cc H_2O). Continue the extraction to a negligible thebaine reaction, but do not try to get the rest of the cryptopine out if it involves loss of codeine. If the CCl_4 test portions are evaporated on the same steam bath where the fumes may affect alkaloidal residues for titration, add a drop of ammonia to each before evaporation to neutralize any acetic acid. The CCl_4 (aside from the test portions) can be saved for redistillation.

52. Continue as in the usual shake out of codeine (E/CN.7/202, paragraph 12). Make the second solution alkaline and pour it back into the first; make the first solution alkaline with an excess of NaOH. Shake out with CCl_4 past three washes: 5 cc alkali solution (second separatory), 5 cc water containing a little ammonium salt and ammonia (third separatory), and a 10 cc wash of water (fourth separatory). Test for completion of the extraction of codeine from each solution in turn by evaporating a small portion of the CCl_4 and testing the residue with Marquis' reagent (2 drops HCHO in 3 cc H_2SO_4).

53. Filter the extracts through a little cotton into a glass dish and evaporate off the solvent. Dry the residue on the steam bath to complete crystallization; cool and weigh. Multiplication by 0.94 gives an approximation of the anhydrous codeine. Dissolve the residue in a little methanol, add 2 or 3 drops methyl red indicator solution, and titrate with fiftieth-normal H_2SO_4, adding CO_2 free (recently boiled) water to obtain the end-point. One cc n/50 acid = .00599 gramme codeine (anhydrous). Since the extraction represents

/4 grammes

4 grammes opium (instead of 2 grammes, as in E/CN.7/202), multiply the result by 100 and divide by 4 for the codeine percentage of the opium as determined on the lime-water extract.

VIII. COLOUR DETERMINATION OF "PORPHYROXINE-MECONIDINE"

54. The chloroform extracts from Stage III of the Method (paragraphs 15-21) may be used for the determination of "porphyroxine-meconidine" instead of codeine. In three separatory funnels put 20, 10 and 10 cc of dilute H_2SO_4 (2 volumes of concentrated H_2SO_4 to 100 of solution). Shake the chloroform extracts with the H_2SO_4 solutions in turn, then combine the H_2SO_4 solutions in the first separatory, pour out a portion of the solution in a test tube and heat in the steam bath to full development of the red colour. Since the "porphyroxine-meconidine" from 4 grammes of opium is in 40 cc aqueous solution, the red colour corresponding to the values previously given may be read on the Lovibond slides with the solution in the 1/8 inch cell, or calculated to correspond to this reading. (See E/CN.7/195; and E/CN.7/202, part VI.)

55. In case it is desired to determine both codeine and "porphyroxine-meconidine", the best way, probably, is to wash the $CHCl_3$ extracts as they are collected, first with water containing a little ammonium salt and a drop of ammonia, and then with plain water; and then evaporate off the $CHCl_3$ and proceed with the residue as in E/CN.7/202, parts IV and VI.

56. It may be mentioned that the chloroform extracts also contain the other phenolic alkaloids (in addition to the "porphyroxine-meconidine") that are extractable by chloroform from alkali. However, a suitable determination has not yet been worked out.

IX. CONCLUSIONS

57. The new features of the new morphine method are the continuous extraction of the alkaloids by ether-acetone and the separation of the other alkaloids from morphine by shaking them out from dichloracetic acid solution with chloroform.

58. In Stage I, (paragraph 11) a lime-water solution of the opium is obtained, as in the present precipitation methods. However, the solvent-extraction method has the advantage that the volume of opium solution need not be so limited, and any of the various methods of obtaining the lime-water solution (paragraphs 28-30) could be used. Furthermore, subsequent stages of the new method are not, in principle, dependent on the use of a lime-water extract, and eventually other methods of extracting the opium can be explored (paragraph 9).

/59. In

59. In Stage II (paragraphs 12-14) continuous extraction with ether-acetone meets a number of objections that have been raised against other extractive methods (paragraphs 5, 6). The great advantage of the new solvent-combination is in extracting so little colour and other extraneous material besides the alkaloids (paragraph 33).

60. In Stage III (paragraphs 15-20), an entirely new method, more successful than any used hitherto (paragraphs 7, 8), is applied to separating the other alkaloids from morphine. This is extraction with chloroform from dichloracetic-acid solution. This separates even the minor phenolic alkaloid which presumably stays with morphine in previous extractive methods (paragraphs 7, 46), carrying along only a negligible amount of morphine (paragraph 47), and leaving behind only a negligible amount of titratable alkaloids extractable by chloroform from alkali solution (paragraph 41).

61. In Stage IV (paragraphs 21-26), as in Stage II, the extraction of morphine is made complete by test. Although the morphine recovered is somewhat brown, it crystallizes completely and the weight, as crystalline morphine, usually corresponds very closely to the titration.

62. In Stage V (paragraph 27) the extracted morphine is titrated with standard acid. For such titrations, an uncertainty as to the exact reading of about 0.1 cc is normal. Every effort has been made to keep any possible error, either positive or negative, within this same limit, and so far as can be ascertained, this has been done (paragraph 47), at least from Stage II onward (paragraph 1).

63. The equivalent of 0.1 cc of tenth-normal acid is 2.85 milligrammes of morphine, or 0.07% of the opium. It is hoped, therefore, that any error from Stage II onward can be kept within about 0.1% of the opium. However, this requires practice and experience (paragraph 40), and the possibilities of greater error, particularly any arising from Stage I (paragraph 38), should not be forgotten (part V, paragraphs 37-41).

64. The method as a whole utilizes the advantages of extractive methods over precipitation methods (paragraph 4), while meeting the principal objections that have been brought against previous extractive methods (paragraphs 5-8). The method is highly flexible and adaptable; no minimum quantity of morphine is necessary, and it can be applied, with no more than obvious modifications, to most kinds of preparations or mixtures of morphine or opium.

/65. The method

65. The method sets a new standard for the assay of opium. It can be used to determine the accuracy of the pharmacopoeial methods (which may seem to have some advantage in simplicity) in the following ways:

(a) The final results can be compared.

(b) The other alkaloids precipitated and titrated with the morphine in a pharmacopoeial method, can be separated as in Stage III of the new method, and determined.

(c) The morphine remaining in the lime-water filtrate in a pharmacopoeial method, can be determined by the new method, starting with Stage II.

(d) The lime-water extracts prepared by different methods can be accurately compared for content of morphine, by means of the new method.

The new separation of the other alkaloids from morphine can also be used to determine whether there is any important error in previous extractive methods, and to modify them so as to avoid a positive error. It is hoped that all these comparative studies will soon be made by international collaboration.

UNITED NATIONS

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11 June 1951
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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

The forms of Crystals in Turkish Opium

The Secretary-General has the honour to publish the following article on the forms of crystals in Turkish opium by Dr. Alâeddin Akçasu which has been contributed by Professor Sedat Tavat, Director of the Institute of Pharmacology, Istanbul University.

This article is a slightly revised edition of one which appeared in the Bulletin of the Faculty of Medicine, Istanbul University, 1950, Vol. 30, No. 3.

THE FORMS OF CRYSTALS IN TURKISH OPIUM

By

Dr. Alâeddin Akçasu

1. Many physical and chemical methods have been proposed for ascertaining the sources of illicit opium.

2. The papers by Charles C. Fulton, submitted to the third session of the Commission on Narcotic Drugs, in 1948, by the representative of the United States of America,[1] referred to the possibility of determining the origin of opium by examining its crystals, and proposed a method. A minute quantity of opium is crushed into an emulsion with a little water on a slide, smeared carefully and left to dry. It is then examined through a polarizing microscope magnifying 80 to 90 times. A 10 per cent solution of NaOH can be added to remove the resins contained in the opium. There is, however, no important difference between the crystals in preparations from which the resin has been removed and in those from which it has not. For this reason either method may be used for examining opium crystals. The examination of various kinds of opium by these methods showed that they contain more crystals than had previously been supposed. Finally, a special method[2] disclosed that the crystals consist of narcotine.

3. Fulton classified opiums according to the forms of these crystals into three groups:[3]

(a) The opium highly crystalline, containing crystals in the form of rods, In this group the field contains chiefly slender, well-formed rods of varying length. This group includes opium from India, China, Korea, and some from Iran.

(b) Instead of slender, well-formed rods this group contains small birefringent particles of varying size. It is represented by opium from Turkey and Yugoslavia.

1/ Document E/CN.7/117, 14 April 1948.

2/ Ibid., part II A, "On determining the Country of Origin of Opium".

3/ Ibid., part II A; also part II C, "Photomicrographs of Opium Crystals".

... inte...
/(c) An

(c) An intermediate group, divided into three sub-groups:

(i) Approaching group (a) but conspicuous rods are thicker and fewer, and most of the birefringent material consistsof tiny rods and irregular particles.
Afghan and some kinds of Iranian opium belong to this group.

(ii) Definitely intermediate: A rew rods are noticeable among many irregular particles over the whole field.
This sub-group includes opium from Mexico, Gwalior and Mongolia, and some from Afghanistan.

(iii) A type resembling the second group: the rods are imbedded in the resin and are not well-formed, and the crystals dispersed by the treatment are small irregular particles or fragments.
Japanese opium belongs to this sub-group.

4. In order to obtain a more exact knowledge of the crystal forms of opiums cultivated in Turkey, we obtained 10-gramme samples of opium from the 1948 crop of the chief opium-producing districts of Turkey.[1/] From these samples we made our preparations. A small quantity of opium was thoroughly crushed on a slide with a few drops of water and the undissolved part removed with the point of a knife. The remaining material is evenly smeared and left to dry. We did not use NaOH solution. We studied these preparations through a microscope enlarging eighty times and took the photographs with E. Leitz Wetzler microphotography apparatus at a magnification of 160 times with a distance of 25 cm. to the ocular.

5. The 14 samples which we examined included some from the districts of Aydin (Figure 1), Acipayam (Figure 2), Çorum (Figure 3), Inegöl (Figure 4), and Akhisar (Figure 5). These have crystals characteristic of the type which Fulton describes as contained in opium grown in Turkey. In these preparations the whole field is covered with irregular birefringent particles of varying sizes, and no rods are seen.

6. In the Aydin opium the birefringent particles are larger than in the other samples. A very few fine dust-like particles can be made out among them.

1/ We obtained our specimens through the Beşiktaş depot by permission of the Department of Agriculture. We desire to thank them both for their valuable and ungrudging assistance.

/7. In the Akhisar

7. In the Akhisar opium large crystals are relatively few, crystals in the form of fine granules predominating in the field.

8. The samples from Acipayam and Inegöl contain crystals of all sizes in equal numbers. In the Inegöl sample, however, separate large crystals with irregular margins were encountered here and there.

9. The crystals of the samples coming from the districts of Sandikli (Figure 6), Zile (Figure 7), Kula (Figure 8) and Uşak (Figure 9) do not conform to the type said to be characteristic of Turkey. They are somewhat similar to Fulton's first type, but an obvious difference is that whereas the crystals of Fulton's first type are in the form of rods with thick, blunt tips, the crystals visible in Figures 6, 7, 8, and 9 appear to be of another type - very fine, sharp-pointed, needle-shaped, and longer.

10. There are also some minor differences between these four samples. For example, in the Uşak opium needle-shaped crystals are in the majority, while in the Sandikli opium they are fewer than the small crystals. In the Zile sample the two kinds are present in more or less equal numbers. In the opium from Kula the needle-shaped crystals are finer and more delicate than in the other samples.

11. The samples from Isparta (Figure 10), Malatya (Figure 11), Yalvaç (Figure 12), Bolvadin (Figure 13) and Beyşehir (Figure 14) are close to the intermediate type. Here, beside a few needle-shaped crystals, there appear fine granular particles.

12. In the Isparta opium the needles are finer and somewhat longer. In the Beyşehir sample they are shorter, and in that from Bolvadin they are very few and resemble rods. In the Malatya opium the crystals are generally fewer, with rods among them here and there.

13. The needles in the Yalvaç opium are longer and somewhat thicker.

14. The sample from Emet (Figure 15) has special characteristics. The whole field is covered with fine, small, well-shaped needles, regularly distributed, and in between them some fine granules appear here and there.

15. To summarize the characteristics we have so far observed: Turkish-grown opiums show not only the single type of crystals mentioned by Fulton, but also two others. In these the crystals are not of rod form but rather needle-shaped. It is thus necessary to recognize each of these samples as of a separate type from his.

/16. If we regard

16. If we regard the Emet opium as a particular type, we may classify Turkish opium thus:

(a) A type consisting of small particles with the crystals irregularly scattered over the field and no crystals of either rod or needle shape. This type corresponds to Fulton's second type.(Figure 1).

(b) (i) A type containing long, fine needles with a small number of irregular particles among them. This type is close to Fulton's first type but the morphology of the crystals is different (Figure 6).

(ii) A type characterized by needle-shaped crystals as in the Emet sample: smaller, well-formed and thickly scattered over the field. A few small particles can be seen among them (Figure 15).

(c) Intermediate. This type is characterized by the presence in the field of view of crystals of both the previous types. The preparations exhibit well-formed needles and irregular particles.(Figure 12).

17. Thus regarded, Turkish opium cannot be classified in a single type, but all the types of crystals we have mentioned in this survey must be borne in mind.

18. We obtained our samples from the places indicated on the map, and those districts have between them no such geographical or climatic relationship as would explain the resemblances or differences in the crystals. These differences found in Turkey may perhaps also exist in other opium-producing countries. As they will not be fully understood until further research has been carried out, this method must be used very cautiously in determining the origin of contraband opium.

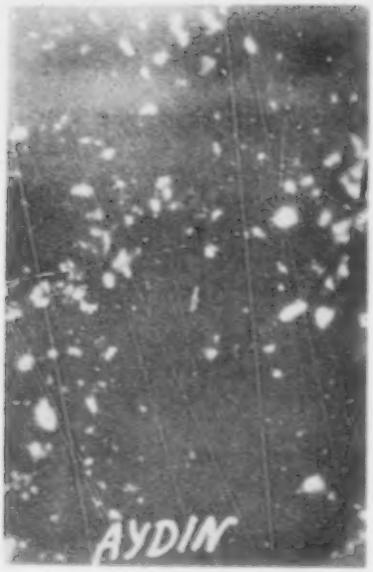

Figure 1

AYDIN

Morphine: 12.50%

Druggist

Irregular crystals of varying size are scattered over the field.

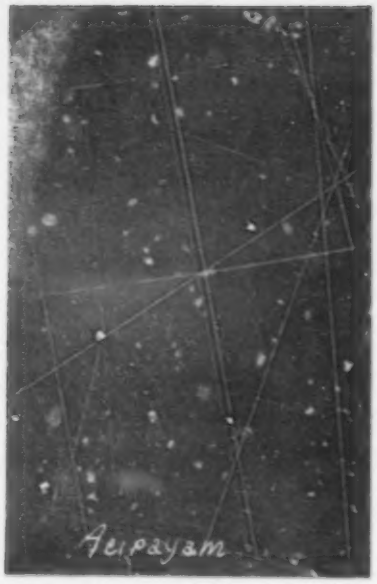

Figure 2

ACIPAYAM

Morphine: 12.50%

Druggist

Smallish crystal particles, scattered, are in the majority

/Figure 3

Figure 3

ÇORUM

Morphine: 13.75%

Soft

Relatively small and scattered particles

Figure 4

INEGÖL

Morphine: 12%

Druggist

A few large, irregular crystals scattered over the field.

/Figure 5

Figure 5

AKHISAR

Morphine: 12.50%

Druggist

Fairly abundant small particles.

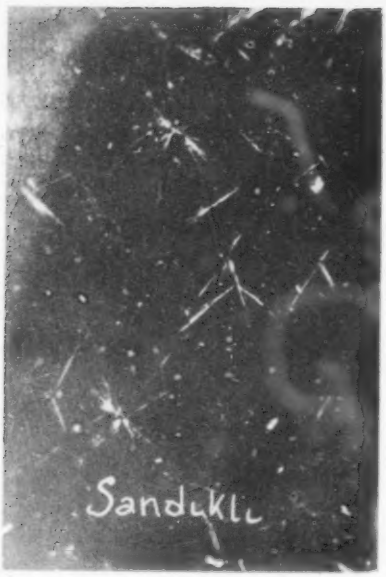

Figure 6

SANDIKLI

Morphine: 10.50%

Druggist

Small particles among long, thin, pointed crystals.

/Figure

Figure 7

ZILE

Morphine: 13%

Soft

Long, blunt-pointed needle-shaped crystals, and irregular particles.

Figure 8

KULA

Morphine: 12.50%

Druggist

A few small particles among thin, fine-pointed needles.

/Figure 9

Figure 9

USAK

Morphine: 13.50%

Druggist

Small particles among long, thin, fine-pointed needles.

Figure 10

ISPARTA

Morphine: 14%

Druggist

Relatively short-thin needles interspersed with small particles.

/Figure 11

Figure 11

MALATYA

Morphine: 9.75%

Soft

One or two small rods, irregular crystals of various sizes and generally of small number.

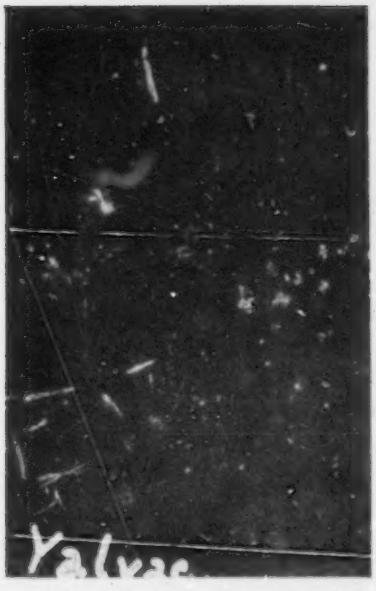

Figure 12

YALVAC

Morphine: 11%

Druggist

A few needles and small crystal particles.

/Figure 13

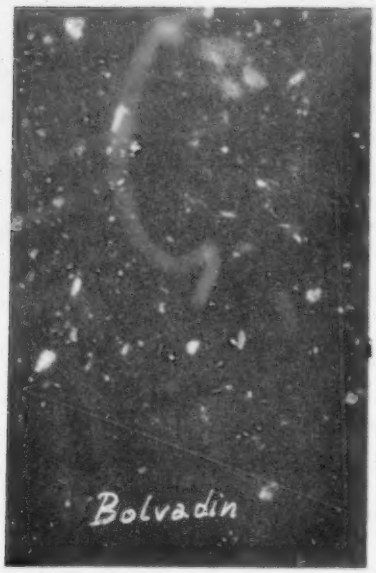

Figure 13

BOLVADIN

Morphine: 12.50%

Druggist

One or two small rods, particles of various sizes.

Figure 14

BEYŞEHIR

Morphine: 12.50%

Druggist

Very small needles interspersed with irregular particles.

/Figure 15

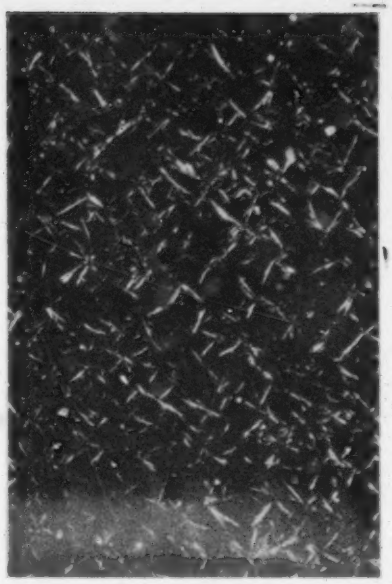

Figure 15

EMET

Morphine: 10%

Druggist

A few small particles among a profusion of small needle crystals.

/MAP

RUSYA
IRAN
IRAK
SURİYE
KARA DENİZ
AK DENİZ
MARMARA DENİZİ
Malatya
Zile
Çorum
ANKARA
Beyşehir
Yalvaç
Isparta
Sandıklı
Uşak
Emet
İnegöl
Kula
Akhisar
Aydın
Acıpayam

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1 August 1951

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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

No. 3

The Determination of morphine in raw opium

by Dr. Erich Knaffl-Lenz

In continuation of the international programme of scientific research on opium, undertaken pursuant to Resolution 246 F (IX) adopted by the Economic and Social Council on 6 July 1949, and the decision of the Commission on Narcotic Drugs at its fifth session, calling for further study of the assay of opium, the Secretary-General is now publishing a method for the determination of morphine in opium, as worked out by Dr. Erich Knaffl-Lenz, former Expert for the Opium Committee of the Health Section, League of Nations.

The Secretary-General wishes to call attention to the following features of the method: preliminary defatting of the opium; total extraction with neutral hot water; and precipitation of troublesome impurities by saturation with sodium sulfate. The method is one of extraction of the morphine by organic solvents; it is not a precipitation method.

THE DETERMINATION OF MORPHINE IN RAW OPIUM

By Dr. Erich Knaffl-Lenz, formerly Expert for the Opium Committee of the Health Section, League of Nations, and Reporter of the Opium Committee of Experts, Office Internationale d'Hygiene publique, Paris

Principle of the method

The preparation of an aqueous extract is made as follows: a part of the content of fats and resins is removed from the opium by means of carbon tetrachloride or a similar fat-dissolving solvent, after which the alkaloids are extracted by neutral hot water, and finally most of the troublesome impurities are precipitated out by saturating the solution with sodium sulfate. Extraction by means of acidified water is not suitable, because the narcotine is also dissolved, the porphyroxine is decomposed with the formation of a red coloring material, and in addition a much greater quantity of impurities goes into solution.

The separation of the morphine from the secondary alkaloids is effected by the addition of a carbonate-free sodium hydroxide solution, by means of which the alkaloids other than morphine and narceine are precipitated. The small quantity of codeine remaining in solution is removed by means of benzene.

The morphine is then liberated from solution by the addition of sodium bicarbonate, and shaken out with a warm mixture of chloroform-ethanol. Since the solubility of ethanol in the saturated sodium sulfate solution is slight, it is quite feasible to obtain the morphine quantitatively, using only small quantities of solvent. The combined extracts are dehydrated with anhydrous magnesium sulfate, then evaporated to dryness, and the morphine residue is then titrated with tenth-normal hydrochloric acid, using methyl red as indicator. When necessary a further purification to lessen the brown colour is made, as described below.

Insofar as the purity of the morphine is concerned, numerous methoxyl determinations have never yielded more than 0.05% of methoxyl, presumably due to a content of narceine, which would be unimportant, since it would not be titrimetrically determined.

This method has the advantage over the usual lime methods of the present time in that the opium is quantitatively extracted, so that neither correction factor nor extract determination is necessary. Moreover, a determination can readily be made in two or three hours.

/Description

Description of the method

3.00 grammes of finely powdered opium, dried at 105° C. are mixed with 20 cc C Cl_4 in a small wide-mouthed Erlenmeyer flask, until a slurry is formed. This is warmed on the water bath with frequent agitation, then poured into a Jena glass sinter filter number 3G3, which has on it a double layer of filter paper, and filtered with suction. The opium is washed twice, each time with 5 cc warm C Cl_4, then air is drawn through long enough for the C Cl_4 to be evaporated.

The dry opium is then brought back into the flask as completely as possible, which can best be done by lifting off the filter paper. It is then treated with 20 cc water, and heated for at least 5 minutes on the steam bath, while being frequently shaken. After this, 2.8 gm Na_2SO_4 are added and dissolved, and the whole is cooled and allowed to stand for at least 20 minutes.

The separation of the opium residue from the aqueous extracts can be carried out either by means of a glass-sinter filter or by centrifuging. In the latter case, however, the centrifugally separated opium residue must be washed onto a filter with 5 cc of saturated Na_2SO_4 solution. The filtration proceeds much more quickly if a small quantity of sea sand is added, which prevents the opium residue from sticking together. When the sinter filter is used it is covered with filter paper, and the liquid is drawn through with slight suction and the residue washed with 5 cc saturated Na_2SO_4. Following the first filtration the opium residue is sucked dry and returned to the flask once more, 20 cc saturated Na_2SO_4 poured over it, and the process just described is repeated.

3 cc 5 n NaOH are added to the filtrate. Another extraction of the opium residue is made with 15 cc Na_2SO_4 solution, and the residue washed with enough sulfate solution to bring the combined filtrates to 75 cc. Most suitable for this filtration is a cylindrical vessel having its upper third constricted to 1 cm diameter, and having markings up to 75 cc. The glass filter is placed over this vessel with a two-holed stopper, the second hole serving as the means of connecting the apparatus with a water-jet vacuum pump.

The combined filtrates, together with the NaOH, are vigorously shaken together, and then after standing 20 minutes, filtered through a folded filter paper into a cylindrical separatory funnel containing 100 cc of warm benzene. This separatory has a total capacity of 200 to 250 cc and is constricted for about 4 cm of its length in the middle to 1 cm. When at least 55 cc of the extract have been filtered through, the separatory is stoppered and then turned

/several times

several times about its axis. This manner of shaking out avoids the formation of troublesome emulsions. The codeine in the solution passes into the benzene.

50 cc of the benzene-extracted solution (corresponding to 2.5 gm of opium) are shaken out for morphine in a separatory funnel of the kind just described. The first shake-out is made with 75 cc of a 3:2 chloroform-ethanol mixture, after adding 15 cc of 8% $NaHCO_3$ solution.

It is advisable to warm the separatory funnel before use by running warm water through it, and also to use warm mixtures, in order to prevent the crystallizing out of Na_2SO_4. If some does crystallize out, it can be dissolved by the addition of a little warm water.

The second shake-out is carried out with 50 cc of 3:1 chloroform-ethanol and the third with 20 cc (3:1). The Marquis reaction can be used to determine the completion of the shaking out.

If small quantities of emulsion should form, it is advisable to draw it off into a small flask, where it can be treated with dry $MgSO_4$, and the filtrate can then be combined with the other shakeouts.

The combined shakeouts are dried by shaking with anhydrous $MgSO_4$, filtered into a small distilling flask, and evaporated to dryness.

If the dry residue is not too dark in colour it can be titrated using methyl red indicator with tenth-normal hydrochloric acid. By multiplying the number of cc of acid used by the factor 1.426 the percentage content of morphine is obtained.

In a number of types of opium the brown colouring matter hinders the titration, in which case the morphine residue is dissolved in the least possible amount of acid, then diluted with 20 cc of saturated Na_2SO_4 solution, and after setting the morphine free it is again shaken out.

Often it suffices to recrystallize the morphine residue as follows: dissolve by warming with 2 cc methanol-benzene (1:3), then add 10 cc of saturated morphine solution, let cool, and after half-an-hour's standing carefully remove the liquid, in which most of the colouring matter remains dissolved, by means of a glass tube drawn out into a capillary. However, the colouring is usually of importance only when larger quantities of opium are used for analysis.

/Comparative Analytical Results

Comparative Analytical Results

Method	Samples				
	Benares	Calcutta	Iran	Turkey	Yugoslavia
Kraffl-Lenz	11.86	11.14	12.08	12.10	16.70
Kier-Wackerlin	11.84	11.27	12.09	12.12	16.62
Harrison and Self	10.47	9.99	11.56	11.46	16.27
International	11.00	10.35	11.52	12.03	16.57

The first two methods give identical values, without the use of a correction factor, whereas the other methods give lower values despite the use of a high correction factor (10 to 16%).

Reagents

1. Anhydrous sodium sulfate.
2. Anhydrous magnesium sulfate.
3. Saturated sodium sulfate solution.
4. 8% sodium bicarbonate solution.
5. Saturated morphine solution.

Preparation: Gradually pour 10 cc of a hot ethanol solution saturated with morphine base into 1 liter of boiled distilled water, with constant stirring and shaking, and after it has been allowed to stand 24 hours, decant the solution from the morphine crystals and keep it in a dark container.

6. 5 normal carbonate-free sodium hydroxide.
7. Chloroform-ethanol mixtures:

   3:2, specific gravity 1.220
   3:1, specific gravity 1.300

The mixtures distilled off can be brought to the right proportions again by adding suitable quantities of ethanol.

8. Benzene.
9. Methanol-benzene, 1:3.
10. Methyl red solution. A solution of 0.05 gm methyl red in 75 cc ethanol is diluted to 100 cc with boiled distilled water.
11. Sea sand, previously cleaned with hydrochloric acid.
12. Carbon tetrachloride.

UNITED NATIONS

SECRETARIAT

GENERAL

ST/SOA/SER.K/3/Corr.1
22 May 1952

ORIGINAL: ENGLISH

THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

No. 3

The determination of morphine in raw opium
by Dr. Erich Knaffl-Lenz

CORRIGENDUM

Page 4, line 3: For "(corresponding to 2.5 gm of opium)" read "(corresponding to 2.0 gm of opium)".

(The factor for calculating the percentage (1.426) was, however, correctly stated for the equivalent of 2.0 grams.)

52-6035

NATIONS UNIES

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11 September 1951

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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

No. 4

"Porphyroxine-Meconidine" in Relation to the Origin of Opium

In continuation of the international programme of scientific research undertaken pursuant to Resolution 246 F (IX), adopted by the Economic and Social Council on 6 July 1949, the Secretary-General is now publishing the present paper, which gives data on the "porphyroxine-meconidine" content of the United Nations opium samples, and conclusions from all the data now available as to the relation between the "porphyroxine-meconidine" content and the origin of the opium.

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/"PORPHYROXINE-

"PORPHYROXINE-MECONIDINE" IN RELATION TO THE ORIGIN OF OPIUM

I. Introduction

1. The purpose of the present paper is to present the new data for "porphyroxine-meconidine" obtained on the United Nations opium samples, and to bring up to date, in the light of this additional information, our knowledge of the relation between the content of "porphyroxine-meconidine" and the origin of the opium.

2. The "porphyroxine-meconidine" is a minor alkaloid of opium which has the property of changing to a red-coloured compound when heated in dilute mineral acid. It is here given this double name because it has been known under both the names "porphyroxine" and "meconidine", while the quotation marks are used because the descriptions of its properties under these names were incorrect in both cases. The history of this alkaloid and some account of its true properties will be found in "The story of 'Porphyroxine-Meconidine', the red-turning alkaloid of opium", an article written simultaneously with this paper, and to be published in the Bulletin on Narcotics, Volume IV, No. 1 (1952). Some information on the true properties in addition to the red-turning characteristics was previously given in document E/CN.7/117, 14 April 1948, part II D, "Some Minor Alkaloids of Opium. Alkaloid 1, 'Porphyroxine-Meconidine'."

3. The Secretariat chemists have previously studied the relation of "Porphyroxine-Meconidine" to origin, using the opium samples then available. (Document E/CN.7/195, 26 March 1950, The Comparative Determination of "Porphyroxine-Meconidine".) The Canadian chemists C. G. Farmilo and P. M. L. Kennett, collaborating in the international opium research, also contributed a study on this subject, using most of the same opium samples (Document E/CN.7/207, 19 October 1950, Further data on "Porphyroxine-Meconidine".) These results were very promising.

4. The new data do not change the general picture, in particular the conclusions that the opium of India is usually especially high in "porphyroxine-meconidine" and that of Iran especially low. It has, however, been found that, although Turkish samples are usually intermediate, some are as high as most of those from India, and a few are very high. All but one of the very high Turkish samples

/were from

were from Zile or Malatya, thus showing a regional difference within the larger region of Turkey. Further discussion of results will be given after the new data, and curves for five of the main regions based on all available data, have been presented.

II. The Method

5. The method used to obtain a comparative colorimetric value for the "porphyroxine-meconidine" was virtually the same as previously given in document E/CN.7/195.

6. Rub 1.5 grammes opium and 1 gramme $Ca(OH)_2$ with 10 cc water to a smooth paste, in a mortar with a pouring lip, then add 50 cc water to the mixture in the mortar and stir frequently during 1/2 hour, getting as complete solution as possible. Filter and put 40 cc of the filtrate (taken as representing 1 gramme of opium) into a continuous extraction apparatus with peroxide-free ether. Extract until the ether coming over will no longer yield a red or pink color when a portion is evaporated on the steam bath in a small porcelain crucible with a few drops of (1:9) HCl. (It is best to stop the boiling while the test portion of ether is taken from the apparatus. The extraction takes about 3 hours with an efficient apparatus).

7. When the extraction is complete, pour the ether into an evaporating dish, rinsing the flask with a little methanol, and evaporate to dryness. Treat the residue with 10 cc 1 per cent HCl (1 per cent by volume of concentrated HCl) and heat on the steam bath, stirring the residue into solution with a glass rod. This develops the red colour. The solution may be evaporated to about 6 to 9 cc during this heating.

8. Pour the solution into a 10 cc graduate and rinse in with just the necessary amount of 5 volume-per cent ascetic acid to make up to 10 cc again. Mix and read the colour in the 1/8 inch cell of the Lovibond tintometer. When the colour is quite weak it may be read in the 1/4 or 1/2 inch cell; when it is very strong it may be read in the 1/16 inch cell, or the solution diluted with 5 volume-per cent acetic acid and then read.

9. After obtaining a reading, pour the solution into a test tube and reheat in the steam bath, cool, and read again. Repeat, if necessary, until the maximum red colour has been found. The colour is matched with the Lovibond red slides, if necessary using some yellow or blue slides. Only the red

/colour

colour value is taken as significant. If the reading is made in a larger or smaller cell than 1/8 inch, or the solution diluted beyond 10 cc, the result is calculated to correspond to the colour developed from 1 gramme opium, in 10 cc solution, read in the 1/8 inch cell. The tabulated results are all on this basis.

III. The United Nations samples

10. The new samples have been given to the United Nations for opium research, by China, France, for Laos and Vietnam, Greece, India, Iran, Korea, Turkey, the United States of America, and Yugoslavia. The sample from the Union of Soviet Socialist Republics listed hereafter was contributed by Laboratoires Clin-Comar, France; and the Iranian export samples by the Mallinckrodt Company, U.S.A.

11. The majority of the samples have been contributions from the producing countries of their own opium, for international distribution to chemists taking part in the opium research, as well as for study by the Secretariat. Some small samples are sufficient only for the Secretariat research. In most cases the large samples received from the producing countries each consisted of several separate lumps or loaves, for example, 4 separate lumps from Merzifon (Turkey No. 7) and 3 from Burdur (Turkey No. 8).

12. For the purposes of this study, each separate lump was run for "porphyroxine-meconidine"; the lumps in the same sample are distinguished as A, B, C, etc. In many cases there were some rather large differences between different lumps included in the same sample. Some of the large samples consisted of quite a number of small lumps or loaves. The majority of these were also run separately; they are distinguished as a, b, c, d, etc.

IV. "Porphyroxine-Meconidine" Values of United Nations Samples

CHINA

U.N. 37 - Received from Taiwan -	lump A	14.0
	lump B	13.5
	lump C	14.4
	lump D	10.5

/GREECE

GREECE

U.N. 25 - small loaf, a	12.0
- b	14.0
- c	13.0
- d	12.8
- e	9.2
- f	9.4
- g	10.0
- h	10.0
- i	11.6
- j	12.0
- k	12.0
- l	12.0
- m	10.4

INDIA

U.N. 34 - Banaras opium	34.6
U.N. 35 - Malwa opium	35.8
U.N. 36 - Excise opium	27.0

IRAN

U.N. 18 - Export opium	5.5
U.N. 19 - Export opium	4.2
U.N. 20 - Export opium	5.0
U.N. 21 - Export opium	4.4
U.N. 47 - Fars	7.0
U.N. 48 - Isfahan	5.6
U.N. 49 - Kerman	5.4
U.N. 50 - Kermanshah	16.2
U.N. 51 - Khorassan	9.0
U.N. 52 - Nehavand	15.3

KOREA

U.N. 16 - Korea No. 1 - A	1.2
No. 1 - B	2.5
U.N. 17 - Korea No. 2	11.6

LAOS

U.N. 26 - Laos, Luang Prabang, lump A	10.0
lump B	20.0
lump C	24.8
U.N. 27 - Laos, Xieng-Khouang, lump A	19.6
lump B	19.2

/TURKEY

TURKEY

U.N. 1 - Turkey No. 1 - Aydin -	lump A	24.4
	lump B	12.4
	lump C	17.2
	lump D	14.4
	lump E	16.4
	lump F	29.6
	lump G	22.0
	lump H	22.0
U.N. 2 - Turkey No. 2 - Afyon -	lump A	25.2
	lump B	18.0
	lump C	7.8
U.N. 3 - Turkey No. 3 - Corum -	lump A	13.0
	lump B	10.0
	lump C	29.6
	lump D	26.0
U.N. 4 - Turkey No. 4 - Cal -	lump A	12.8
	lump B	8.0
	lump C	7.2
	lump D	14.2
U.N. 5 - Turkey No. 5 - Usak -	lump A	10.8
	lump B	18.4
	lump C	21.0
	lump D	2.8
U.N. 6 - Turkey No. 6 - Nallihan -	lump A	32.0
	lump B	10.0
	lump C	11.2
	lump D	28.0
	lump E	12.0
U.N. 7 - Turkey No. 7 - Merzifon -	lump A	5.6
	lump B	9.0
	lump C	14.4
	lump D	14.8
U.N. 8 - Turkey No. 8 - Burdur -	lump A	5.3
	lump B	18.0
	lump C	7.8
U.N. 9 - Turkey No. 9 - Kula -	lump A	24.8
	lump B	23.9
	lump C	13.0
	lump D	23.4

/U.N. 10 -

U.N. 10 - Turkey No. 10 - Sandikli	lump A	14.0
	lump B	32.6
	lump C	10.0
U.N. 11 - Turkey No. 11 - Aksehir	lump A	15.2
	lump B	22.4
	lump C	44.0
U.N. 12 - Turkey No. 12 - Zile	lump A	16.8
	lump B	40.0
	lump C	64.0
	lump D	20.2
	lump E	31.0
	lump F	48.8
U.N. 13 - Turkey No. 13 - Isparta	lump A	15.0
	lump B	16.0
	lump C	8.4
U.N. 14 - Turkey No. 14 - Malatya	lump A	70.8
	lump B	41.2
	lump C	23.2
	lump D	72.0
	lump E	64.8
U.N. 15 - Turkey No. 15 - Export opium		11.6
U.N. 29 - Exhibition	A, half loaf	11.8
	B, whole loaf	10.0
U.N. 30 - Exhibition, standardized opium		12.4
U.N. 31 - Exhibition, reduced size cubes		13.6
U.N. 40 - Purchased at Eskiser		4.1
U.N. 41 - Purchased at Kutahya	ball-A	15.2
	lump B	8.0
U.N. 42 - Purchased at Afyon		3.2
U.N. 44 - Loaf in copper vessel		9.0

UNION OF SOVIET SOCIALIST REPUBLICS

U.N. 39 - Sample from France		10.0

/VIETNAM

VIETNAM

U.N. 28 - Tonkin, Laokay,	lump a	4.3
	lump b	20.2
	lump c	15.2
	lump d	13.8
	lump e	13.8
	lump f	16.4
	lump g	12.0
	lump h	37.0
	lump i	20.0
	lump j	18.4
	lump k	13.8
	lump l	14.4
	lump m	10.8
	lump n	16.4
	lump o	20.0
	lump p	21.0

YUGOSLAVIA

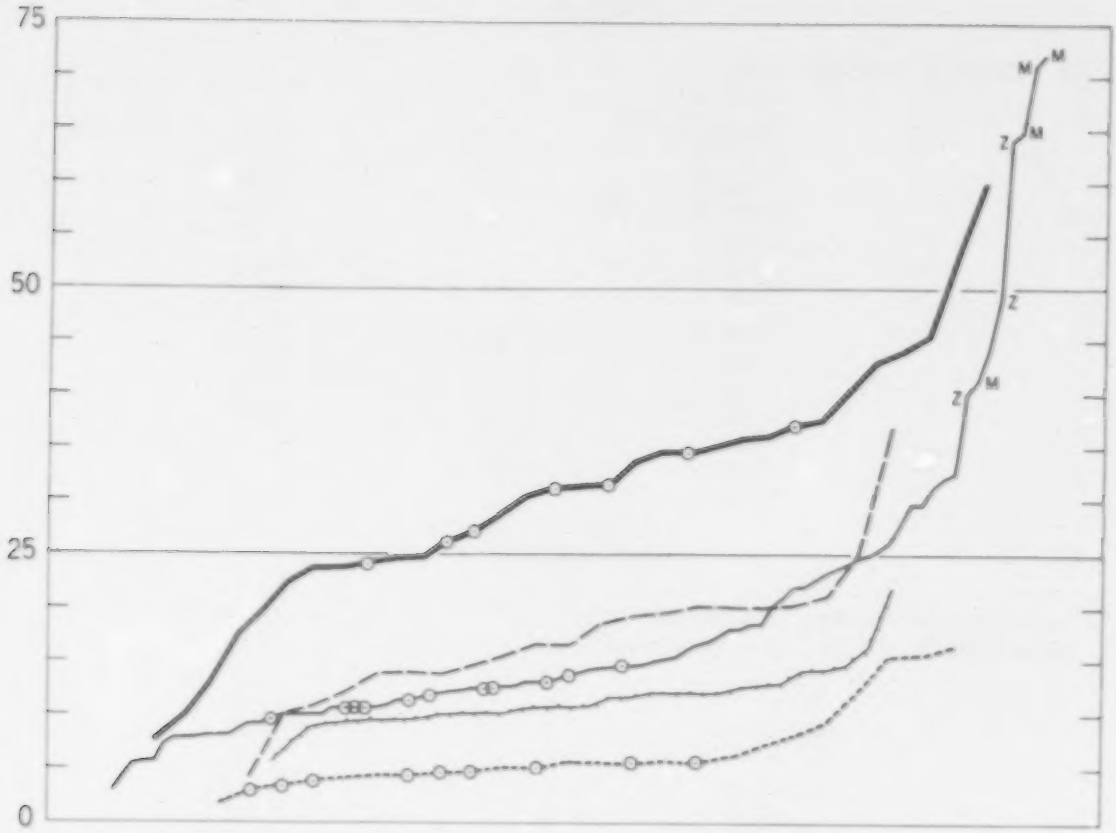
U.N. 24 - Yugoslavia No. 1 - Export type		12.4
U.N. 38 - Yugoslavia No. 2 - small loaf,	a	14.0
	b	16.0
	c	21.8
	d	10.8
	e	10.8
	f	10.8
	g	10.0
	h	19.6
	i	10.8
		10.8

V. Chart of "Porphyroxine-Meconidine" Values

14. The accompanying chart shows all the available data for five of the main regions of opium production, and shows the comparative results. The curves represent 88 samples from Turkey, 32 from India, 30 from Greece and Yugoslavia, 24 from Iran, and 21 from Laos and Vietnam. Horizontally, the samples are simply arranged in their order of value for each curve, and the values are spaced more widely the fewer the number of samples. The ordinates correspond to the colour values obtained on the various samples.

/Chart

COMPARATIVE "PORPHYROXINE-MECONIDINE" VALUES IN FIVE PRODUCING REGIONS



Turkish samples are spaced 1 unit apart, Yugoslavia and Greece 2, India 2 1/2, Iran 3, Laos and Vietnam 3.

Turkey - 88 samples, Yugoslavia and Greece - 30, India - 32, Iran - 24, Laos and Vietnam - 21.

Ordinates correspond to the colorimetric "porphyroxine-meconidine" values.

Circles indicate samples of export opium of India, Iran, and Turkey.

"Z" and "M" indicate samples from Zile and Malatya (Turkey).

VI. Facts and Conclusions from all the Data

15. Conclusions must be based, not merely on the new data, but on all the available data. The chart given in paragraph 14 shows the comparative values for all samples thus far available from five of the principal producing regions. The generally high trend of values for samples from India, and the generally low trend of values for samples from Iran, are clearly apparent. Some of the Turkish samples are high and a few are very high, but in general the samples from Turkey, as well as most of those from Greece and Yugoslavia, and Laos and Vietnam, have intermediate values. Most samples from other countries, not shown on the chart, are also in the intermediate range. The specific values for all the earlier samples will be found in document E/CN.7/195.

16. Export samples of India, Iran and Turkey are marked on the curves with circles. The export opium of Turkey is made by mixing all the opium of the country, and thus in itself represents the average of Turkish opium. The export samples of Turkey fall almost entirely on the lower half of the curve for Turkey as represented here. The significance of this is that in obtaining representative samples of different types, the varieties of Turkish opium containing high "porphyroxine-meconidine" have been over-represented, in comparison with the quantity of production. The Turkish export samples (values 9.4 to 14.4) indicate virtually the same curve as found for Balkan opium (Greece and Yugoslavia). This is to be expected for Turkish "druggist" opium, which constitutes the chief production of the country and is known to be quite similar to Balkan opium.

17. The "porphyroxine-meconidine" values may be broadly divided into four zones: low, up to 8; intermediate, 8 to 22; high, above 22; very high, above 34. The main part of the curse for Iran is below the value 8; the main parts of the curves for Greece and Yugoslavia, Laos and Vietnam and Turkey, (as well as the values for most other countries whose samples have been tested), are between the values 8 and 22; the main part of the curve for India is above the value 22. A significant part, though not the main part, of the Turkish curve is still above this value, and also a very few scattered values from other countries.

18. About one-third of the curve for India is in the "very high" zone, above the value 34. Also a few samples from Turkey rank with, or even above, the highest of the Indian samples: all but one of these were from Zile or

/Malatya,

Malatya, thus showing a regional difference within the larger region of Turkey. The one other Turkish sample having a very high value was from Aksehir (value 44.0). Only two samples neither from India nor from Turkey have been found so far with values above 34: one of these was from Vietnam (Tonkin, value 37.0) and the other (value 67.2, not shown on the chart) was from Japan, where opium is no longer produced.

19. The Indian and Iranian opiums are in several respects quite similar, but in "porphyroxine-meconidine" values they are almost always entirely different. In determining the origin of contraband opium this test is valuable, especially in distinguishing Indian and Iranian opiums from each other, and to some extent in distinguishing either Indian or Iranian opium from most other kinds. The possibility of a sample with a very high value being Turkish rather than Indian, and the possibility of a very low value resulting from adulteration or the inferior grade of a particular opium rather than indicating Iranian origin, should always be kept in mind. In the latter case the ratio of "porphyroxine-meconidine" value to morphine percentage may mean more than the "porphyroxine-meconidine" value taken alone.

20. The "porphyroxine-meconidine" colour reading is very valuable in determining the origin of seized opium, but it cannot be decisive in itself. It must be considered in conjunction with other tests, or in connexion with the facts and circumstances of the seizure. Rightly used, it is one of the best tests for the chemical determination of origin. The different positions of the curves for various countries, and in particular the consistent differences between the export opiums of India, Iran and Turkey are eloquent of the relation of "porphyroxine-meconidine" value to origin.

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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

No. 5

The Ratio of Narcotine to Meconic Acid in Relation to the Origin of Opium (Preliminary Report from the Government Laboratory, London, England)

By J.R. Nicholls and E.G. Kellett

In continuation of the international programme of scientific research undertaken pursuant to resolution 246 F (IX), adopted by the Economic and Social Council on 6 July 1949, the Secretary-General is now publishing three articles, Nos. 5, 6, and 7 in this series, on the determination of narcotine and meconic acid in opium. These three articles present three different methods successively worked out at the Government Laboratory, London, England. While the third method to a great extent supersedes the first two, the Secretary-General considers that the first two methods include features which may be suggestive and helpful to the chemists working on opium, and that they are so different from the third method as to warrant separate publication. Moreover, the substances hindering complete precipitation of narcotine or meconic acid are also dependent on the type of opium and its origin. Thus, although the first method, which is presented in this paper, was hardly more than preliminary, it yielded results more obviously indicative of origin than the methods later developed which are more accurate for narcotine and meconic acid. For this reason the present method still deserves consideration in relation to the origin problem.

THE RATIO OF NARCOTINE TO MECONIC ACID IN RELATION OF THE ORIGIN OF OPIUM

(Preliminary Report from the Government Laboratory, England)

By J.R. Nicholls and E.G. Kellett

1. Work at first was carried out on extracts of opium in N/5 hydrochloric acid (5 g. opium to make 50 ml of extract) with the object of getting all the alkaloids and meconic acid into solution, with a minimum of gummy matter.

2. The most promising early results were based on two observations:

   (a) When the extract is adjusted to 30-40% alcohol by volume and saturated with $NaHCO_3$, narcotine crystallizes out nearly pure.

   (b) When the mother-liquor from this separation is made strongly alkaline with NaOH, trisodium meconate crystallizes out. This salt titrates as a mono-acid base to brom-phenol blue.

3. Figures for narcotine and meconic acid obtained in this way are not absolutes. Narcotine has a small but not wholly negligible solubility in 40% alcohol. The solubility of trisodium meconate in aqueous alcohol is considerable, but is much reduced by the presence of excess NaOH. By working in specified conditions on different opium samples, however, figures for the narcotine/meconic-acid ratio have been obtained, which appear to be reasonably characteristic of any one of the types examined, and sufficiently different between different types to constitute a ground for diagnosis. Advantages of the procedure are its simplicity, and the fact that, since the diagnostic figure is a ratio, no confusion can be caused by an unusual amount of water or other inert diluent in the opium.

4. Results obtained were as follows:

	Sample	Narcotine/Meconic-acid (in 40% ethanol)
Turkey	1	.69
"	2	.70
"	3	.73
"	4 (League of Nations IV)	.76
India	1 (Ghazipur)	2.03
"	2 (")	2.33
"	3 (Malwa)	2.02
"	4 (League of Nations I)	2.65
"	5 (League of Nations II)	2.90
Yugoslavia	1	1.28
"	2 (League of Nations V)	1.24
Iran	1	1.52
"	2	1.37
"	3	1.41
"	4 (League of Nations III)	in 50% methanol 1.38
		in 33% ethanol 1.60

/5. The apparent

5. The apparent spread is comparatively wide on the Indian samples where the meconate figure is lowest, because the solubility of trisodium meconate makes a proportionately large effect on a small figure; but, as far as we have gone, it appears that any narcotine/meconic-acid ratio greater than 2 is evidence of Indian origin.

6. With regard to the Iranian opium "League of Nations III", see the Experimental Part, paragraph 11.

EXPERIMENTAL

7. About 5 gm. opium are weighed out, and triturated in a mortar with 20 ml N/5 HCl. The liquid is poured into a stoppered vessel, followed by two further 10 ml portions of N/5 HCl which are worked in the mortar so as to leave it finally clean. The vessel is shaken at intervals for three hours or more, and the contents filtered with suction (a filter-aid is sometimes of advantage). The solid matter is stirred with 10 ml fresh N/5 acid and filtered into the first filtrate.

8. To the filtrate is added 2/3 of its volume of ethanol, and $NaHCO_3$ until a little remains undissolved. The liquid is stirred at intervals for an hour or more, and filtered; about 1 gm. of solid NaOH is added to the filtrate and stirred until dissolved, and the liquid is set aside for 24 hours.

9. The solid residue from the $NaHCO_3$ treatment is washed with 40% ethanol until the washings are colourless, then transferred to an open dish and dried by evaporating down with acetone. It is then extracted with three 10 ml portions of cold chloroform. The filtered chloroform extract, evaporated and dried at 80°C, yields white crystalline narcotine, m.p. usually 170° (pure narcotine 174°).

10. The liquid containing the NaOH, after standing, is filtered; the solid residue is well washed with 4:1 acetone-water, and should be a granular powder of a clean lemon-yellow, dissolving in water to a clear yellow solution which is titrated with N/10 acid to bromphenol blue. Meconic acid = titration x 0.020.

11. Of the fifteen authenticated samples we have so far been able to obtain, only one gave any trouble, namely the Persian "League of Nations III".

In the meconate separation, this gave a copious brown sticky deposit which obviously could not be treated as pure trisodium meconate. A satisfactory product was obtained by working with extract: ethanol 2:1 instead of 3:2. This would probably be satisfactory in all cases.

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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

No. 6

The Determination of Narcotine and Meconic Acid in Opium. Second Method. (A Report from the Government Laboratory, London, England)

By J.R. Nicholls and E.G. Kellett

In continuation of the international programme of scientific research undertaken pursuant to resolution 246 F (IX), and adopted by the Economic and Social Council on 6 July 1949, the Secretary-General is now publishing three articles, Nos. 5, 5, and 7 in this series, on the determination of narcotine and meconic acid in opium. These three articles present three different methods successively worked out at the Government Laboratory, London, England. While the third method to a great extent supersedes the first two, the Secretary-General considers that the first two methods include features which may be suggestive and helpful to the chemists working on opium, and that they are so different from the third method as to warrant separate publication. The present paper gives the second of these methods.

The Determination of Narcotine and Meconic Acid in Opium

Second Method

By J.R. Nicholls and E.G. Kellett

A report from the Government Laboratory, England

I. SYNOPSIS

1. The opium is extracted with cold 2 per cent acetic acid. Narcotine and barium meconate, with other constituents, are precipitated from the extract by making slightly alkaline in the presence of barium chloride. The narcotine is washed out of the precipitate with acetone, purified by precipitation from the acetone solution with dilute sodium hydroxide, and redissolved in acetone. The acetone solution is finally filtered, evaporated and dried at 100°C.

2. The acetone-washed precipitate from the opium extract is treated with dilute sulphuric acid to convert barium meconate into meconic acid, which is then brought into solution by adding sodium bicarbonate. This solution, after filtration, is made more strongly alkaline so as to form the sparingly soluble trisodium meconate which is precipitated by saturating with sodium chloride. The trisodium salt, after filtering and washing with aqueous alcohol saturated with sodium chloride, is determined by titration as a mild alkali to brom-phenol blue.

II. DISCUSSION

3. For the extraction of narcotine and meconic acid from opium, 2 per cent acetic acid appears the most suitable solvent of those which have been tried. It causes no radical decomposition, and dissolves only minimal amount of resins and neutral substances; it is sufficiently acid to bring the basic alkaloids, including narcotine, into solution, but not so acid as to repress the solubility of meconic acid as mineral acids do.

4. Narcotine, being a feebler base than the other preponderant alkaloids in the extract, is accordingly precipitated at a lower pH. The point of neutrality to cresol red (pH about 8) has been found suitable in practice. Adjustment to this pH in the presence of alcohol (about 40 per cent) will indeed precipitate the narcotine from an opium extract in sufficient purity to appear as well-formed and characteristic crystals. The presence of alcohol, however, involves a positive, though small, residual solubility of narcotine, and is also an inconvenience in causing meconates to precipitate in a gelatinous form which is not easily washed.

/The narcotine

The narcotine is therefore precipitated in slightly cruder form from aqueous solution only, and is easily purified by making use of its ready solubility in acetone and insolubility in dilute sodium hydroxide solution.

5. The opium extract is liable to contain some calcium salts, which at pH8 would bring down some meconic acid as calcium meconate. Barium chloride is therefore added, to ensure the precipitation of substantially the whole of the meconic acid at this stage. No conditions have been found in which the mother-liquor does not give a distinct meconic acid reaction with ferric chloride. Precipitation as the lead salt appears to be more complete; the difference between the two however has in quantitative terms been found to be very small, the ferric chloride reaction being a highly sensitive one; and precipitation with barium gives a product so much less slimy and more workable that this method is for practical purposes preferred.

6. Trisodium meconate, in which the hydrogen of a hydroxyl group as well as two carboxyl groups is replaced by sodium, is a yellow salt of characteristic appearance, sparingly soluble in water on the alkaline side of neutrality. Its solubility may be further repressed by the presence of alcohol, and if it is desired to prepare and dry the pure salt, this is often a practicable procedure. In some cases, however, the presence of alcohol causes precipitation of trisodium meconate from an opium extract in a gelatinous form which is very difficult to wash free from the mother-liquor. For purposes of determination without isolation, therefore, it is preferred to repress the solubility by increased concentration of sodium ion (saturation with sodium chloride); as the volume of solution, following on concentration of the meconic acid in the form of the barium salt, can be kept quite small, the residual solubility effect is in fact neglibible. The salt thus precipitated can be easily washed with alcoholic brine until contaminated only with sodium chloride, and then determined by titration to brom-phenol blue (conversion of trisodium to disodium salt).

III. EXPERIMENTAL PROCEDURE

7. Extraction. 5 g. opium are triturated with 2 ml acetone, which facilitates the breaking down of sticky lumps and, drying off during the process, leaves the opium as a friable powder readily accessible to the action of the solvent.

8. The powdered material is well shaken with 40 ml 2 per cent acetic acid, and left with occasional shaking for some hours. 1 g.of a siliceous filter-aid is shak

/shaken in,

shaken in, and the mixture filtered with suction. The solid residue is returned to the extraction vessel; with its admixture of filter-aid, it should detach cleanly from the funnel and paper. The paper is rinsed with 20 ml of 2 per cent acetic acid, which is then used for a further extraction of the solid residue. This procedure is followed, without further additions of filter-aid, for two or three further extractions with 20 ml portions of 2 per cent acid. The last filtrate should show not more than a slight meconate reaction with ferric chloride.

9. A convenient way of applying this reaction as a test in the presence of acetates etc. is to make up a 10 per cent solution of sodium acetate, add ferric chloride until a strong colour is developed, then add a measured amount of conc. hydrochloric acid from a burette until the colour is discharged and the solution remains only a pale greenish yellow. Then add further hydrochloric acid equal to one-tenth of the volume already added. Treatment of a portion of this reagent with one-tenth of its volume of a solution containing acetate will only restore the proportions at which no acetate colour is developed, and any marked red or purple colour may be regarded as due to meconate. The reaction is so sensitive that a slight colour is of no practical quantitative significance, and to continue extraction of the opium until the reaction is entirely negative would be no more than an unprofitable dilution of the extract.

10. Separation of narcotine. To the combined extracts, 2 g. barium chloride, dissolved in a little hot water, is added. 10 per cent sodium hydroxide solution is then gradually added until a drop of the liquid reacts definitely pink to cresol red. The extract is sufficiently a buffer in its properties to make the colour-change quite gradual and easily controlled. The mixture is well-stirred and allowed to stand, preferably overnight.

11. 1 g. of filter-aid is stirred in, and the solid filtered with suction, washed with water until the washings are colourless, and pressed and sucked as dry as possible. The solid is extracted by several times breaking up the cake with a little acetone, and sucking through into a clean receiver. Care should be taken to counter creeping of narcotine up the side of the funnel. A total volume of more than about 25 ml acetone is unnecessary and undesirable.

12. The acetone solution is precipitated by the addition of ten times its volume of water, and allowed to stand, with occasional stirring, until the supernatant liquid is clear. 0.5 g. of sodium hydroxide, dissolved in water, is then stirred in; the solid narcotine is filtered off, washed with water until the washings are

/neutral, then

neutral, then washed through with acetone into an open vessel in which the acetone can be evaporated and the residue dried and weighed. The solid narcotine thus finally obtained should be colourless and well crystallized, with a melting-point not below 172°C. In a doubtful case, it may be purified by taking up in a little acetone and repeating the precipitation. With pure narcotine, the loss due to such treatment is less than 0.01 gm. provided that precipitation with water is effected before the addition of alkali, though direct addition of caustic alkali to the acetone solution may cause loss of narcotine by opening of the lactone ring.

13. Separation of trisodium meconate: The original precipitate from the opium extract, after washing out the narcotine with acetone, is washed with a little alcohol and alcohol-chloroform to remove possible small amounts of other alkaloids. It is then removed from the filter and broken up with 10 ml N. sulphuric acid. After standing for an hour, 1 g. sodium bicarbonate is stirred in, and the mixture filtered. The solid residue is washed with two portions each of 7-8 ml water. To the filtrate and washings, 2 ml N. sodium hydroxide are added, and solid sodium chloride is gradually stirred in until a little remains undissolved. After standing for a few hours, the precipitated trisodium meconate is filtered off and washed with 50 per cent aqueous alcohol, saturated with sodium chloride, until the washings are colourless and neutral to phenolphthalein. The residue should be a granular powder of a clear lemon-yellow, and when dissolved in 20 ml N/10 sulphuric acid should show little or no brown or red colour; it may then be back-titrated to brom-phenol blue with N/10 sodium hydroxide. 1 ml N/10 acid is equivalent to 0.020 g. meconic acid. If the solution before titration is noticeably discoloured, re-precipitation by making alkaline and again salting out is an effective means of purification, and involves no significant loss.

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No. 7

The Determination of Narcotine and Meconic Acid in Opium. Third Method.

(A Report from the Government Laboratory, London, England)
By J. R. Nicholls and E. G. Kellett

In continuation of the international programme of scientific research undertaken pursuant to resolution 246 F (IX), adopted by the Economic and Social Council on 6 July 1949, the Secretary-General is now publishing three articles, Nos. 5, 6, and 7 in this series, on the determination of narcotine and meconic acid in opium. These three articles present three different methods successively worked out at the Government Laboratory, London, England. The Secretary-General has considered that the first two methods include features which may be suggestive and helpful to the chemists working on opium, and that they are so different from the third method as to warrant separate publication. However, the third method, which is the present paper, to a great extent supersedes the first two. It is believed to be the easiest of the three to carry out, and also to give the most accurate results.

The Determination of Narcotine and Meconic Acid in Opium, Third Method.

by

J. R. Nicholls and E. G. Kellett

A Report from the Government Laboratory, England

Summary

1. Narcotine present in opium as free base or as a readily dissociated salt is quickly and cleanly extracted by cold acetone.

2. All the narcotine not extracted as under 1. is easily extracted by subsequent treatment with water only.

3. From a neutral or slightly alkaline aqueous solution of a narcotine salt, narcotine is precipitated quantitatively on saturation with sodium acetate. In the presence of 25 per cent of ethanol or methanol, such precipitation, though not complete, is nearly so; and, in this case, even in the presence of other opium extractives, the narcotine separates in crystalline and substantially pure state.

4. Extraction of meconic acid from opium by direct treatment with water is slow and incomplete. With dilute acetic or mineral acids (about N/5) extraction is complete but still slow. After exhausting the opium with acetone, however, the meconic acid can be extracted in warm water without undue difficulty, and in a purer state than by acid extraction.

5. From slightly alkaline solutions, meconic acid is precipitated by barium ion as barium meconate.

6. From solutions alkaline to phenolphthalein, trisodium meconate can be almost completely precipitated by salting out with sodium ion. Trisodium meconate titrates as a mono-acid base to bromphenol blue.

Discussion

7. Most of the narcotine in opium is easily extracted by acetone, unaccompanied by any notable amount of other extractives except for a neutral gummy material insoluble in acids and hence easily separated from the narcotine. With some opiums it has been found that, even after exhaustive percolation with acetone, a small amount of narcotine remains in the opium, from which it is then easily extracted with water. It seems possible that this may correspond to the presence of narcotine partly as easily-dissociated salt such as meconate, and partly as a

/salt of

salt of a stronger acid such as sulphuric. This point is under further investigation.

8. For purposes of determining the total narcotine, that present in the aqueous extract is precipitated by addition of sodium acetate; narcotine is washed out of this precipitate with acetone and added to the main portion already obtained by direct acetone extraction.

9. A rapid though not perfectly complete separation of the narcotine from opium may be effected by extracting it with dilute acid, adding to the extract one third of its volume of ethanol or methanol, and stirring in sodium bicarbonate, until a little remains undissolved. Narcotine separates in crystals and is determined by filtering these off, washing with dilute caustic alkali and with water, then washing through with acetone and evaporating with acetone solution.

10. When narcotine is precipitated with sodium acetate from the aqueous extract of opium, as described above, a little calcium meconate may also be precipitated and remains undissolved on subsequent washing with acetone. The main bulk of the meconate is precipitated by barium chloride. From the calcium and barium meconate combined, meconic acid is liberated by the action of a small volume of dilute sulphuric acid; and from the resulting solution trisodium meconate is precipitated and titrated as indicated in the Summary, paragraph 6. The volume of solution can be kept so small as to render the solubility correction insignificant.

11. Representative results obtained by the above procedure are:

	% Narcotine	% Meconic acid
India - League of Nations 1935, I	4.5	2.0
" " " " II	5.7	3.0
Iran " " " " III	7.8	5.0
Another sample	7.4	5.4
Turkey - League of Nations 1935 IV	4.4	4.3
Another sample	2.95	3.0
Yugoslavia - League of Nations 1935 V	3.5	5.5
Another sample	2.4	3.4

12. It appears possible that the narcotine/meconic acid ratio may be a characteristic of the type of opium and it is hoped to give further attention to this point when more figures for "total" and "acetone-soluble" narcotine have been obtained.

/Experimental

Experimental:

I. Extraction of narcotine and meconic acid from opium

13. 5g. of opium are triturated with 2 ml acetone which facilitates the breaking down of sticky lumps and, drying off during the process, leaves the opium as a friable powder readily accessible to the action of extracting solvents.

14. The powdered material is well stirred with 30 ml acetone and allowed to stand for an hour. The mixture is then filtered with suction, and the residue percolated with a further 20 ml acetone. The combined acetone portions are evaporated to dryness, and extracted by thorough stirring with dilute hydrochloric acid (20 ml of N/2 is a convenient amount) and subsequent washing with water. To the filtered extract, N.NaOH is gradually added until a permanent precipitate just forms. A few gm. of sodium acetate are then stirred in and the mixture allowed to stand until the supernatant liquid is clear. The solid is filtered off under pressure, washed with dilute NaOH and with water, sucked as dry as possible and finally washed through with acetone into a dry receptacle in which it is evaporated and weighed after drying at 100°.

15. The opium residue from the acetone extraction is shaken with successive portions of 30-40 ml of water at about 60° until the aqueous extract shows not more than faint turbidity with barium chloride solution (normally three portions suffice).

II. Precipitation of narcotine and of trisodium meconate

16. Sodium acetate is stirred into the filtered aqueous extract until it is alkaline to methyl red. After standing until the supernatant liquid is clear, the solid is filtered off and washed with a little water. To the filtrate, 1-2g barium chloride dissolved in a little hot water is added. The solid is washed with about 25 ml acetone and the residue reserved for treatment along with the barium meconate precipitate. The acetone washings are diluted largely with water, and the precipitated narcotine filtered off, washed with dilute NaOH and evaporated and dried as before. The weight of narcotine so obtained, added to that obtained under I. above, represents the total narcotine in the opium.

17. The precipitate formed by adding barium chloride to the filtered aqueous extract is filtered off and washed with a little water, and the small insoluble residue from the narcotine precipitation added to it as noted above. It is then broken up with 10 ml N. Sulphuric acid. After standing for an hour, 1g of

/sodium

sodium bicarbonate is stirred in, and the liquid filtered off. The residue is washed with two portions each of 7-8 ml water.

18. To the filtrate and washings, 2 ml N. sodium hydroxide are added, and solid sodium chloride is gradually stirred in until a little remains undissolved. After standing for a few hours, the precipitated trisodium meconate is filtered off and washed with 50 per cent aqueous alcohol, saturated with sodium chloride, until the washings are colourless and neutral to phenolphthalein. The residue should be a granular powder of a clear lemon-yellow and when dissolved in 20 ml N/10 sulphuric acid should show little or no brown or red colour; it may then be back-titrated to brom-phenol blue with N/10 sodium hydroxide; 1 ml N/10 acid is equivalent to 0.020g meconic acid. If the solution before titration is noticeably discoloured, reprecipitation by making alkaline and again salting out is an effective means of purification and involves no significant loss.